THE MECHANISM OF SYNERGISTIC ACTION OF DMC WITH DDT AGAINST RESISTANT HOUSE FLIES

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With the recent widespread occurrence of house fly resistance to many of the halogenated hydrocarbons, intensive efforts have been made to find chemicals which might enhance the effectiveness of these insecticides, especially DDT, against resistant house flies.

In the course of searching for such materials, Perry and Hoskins (1950, 1951a) found that piperonyl cyclonene increased markedly the effectiveness of DDT against various strains of DDT-resistant house flies. Sumerford, Goette, Quarterman and Schenck (1951) reported on the potentiation of DDT against resistant house flies by several structurally related compounds including 1,1-bis-(*p*-chlorophenyl) methyl carbinol (DMC). In a later work, Sumerford, Fay, Goette and Allred (1951) discussed the results of preliminary screening of several other candidate synergists for DDT. More recently, March, Metcalf and Lewallen (1952) have added to this list several effective synergists for DDT and closely related compounds.

Although a substantial amount of data has been reported on the potentiation of various insecticides by so-called synergists, little attention has been given to the biochemical significance of this phenomenon.

Various explanations have been advanced regarding the mode of action of synergistic compounds. David and Bracey (1944) attributed the action of several synergists in pyrethrum fly sprays to a delay in the knockdown of test insects, resulting in longer flying periods and in accumulation of larger amounts of the toxicant. Parkin and Green (1944), on the other hand, showed that retardation of knockdown was not a factor in synergistic action. Dove (1947) concluded that pyrethrum residues are stabilized in thin films by certain synergists, particularly piperonyl butoxide. Munro (1942), Lindquist, Madden and Wilson (1947) and others suggested that pyrethrum synergists may assist the penetration of the toxicant through the insect cuticle. Page and Blackith (1949) proposed that a loose molecular complex is formed between pyrethrins and synergists, and that the presence of those synergists influences the orientation of the pyrethrin molecules at the nerve-sheath interface, resulting in a greater concentration of the toxicant at the site of action. The observations made by Wilson (1949) on the action of piperonyl butoxide and piperonyl cyclonene with pyrethrum on house flies led him to conclude that the synergists damage a detoxifying mechanism. The detailed studies of Chamberlain (1950) on the mode of action of piperonyl butoxide with pyrethrins indicated that the enzyme lipase, obtained from roach and house fly extracts, was, in part, responsible for the detoxification of pyrethrins, and that inclusion of piperonyl butoxide in the formulation produced some degree of inhibition

of the hydrolytic action of lipase. More recently, Perry and Hoskins (1950, 1951b) demonstrated that the synergistic action with DDT imparted by piperonyl cyclonene was not the result of increased permeability of the house fly cuticle but was largely due to inhibition of detoxification of DDT.

The purpose of this report is to give a detailed account of the effect of 1,1-bis-(*p*-chlorophenyl) methyl carbinol (DMC) as a synergist for DDT, and to show a quantitative relationship between synergistic action and inhibition of DDT detoxification in resistant house flies.

Materials and Methods

The following materials were used in this study:

*p,p'*DDT; 2,2-bis-(*p*-chlorophenyl)-1,1,1-trichloroethane, recrystallized twice from ethanol; m.p. 108° C.

*p,p'*DDE; 2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethylene, obtained by dehydrochlorination of DDT in alcoholic KOH solution; m.p. 88° C.

*p,p'*DMC; 1,1-bis-(*p*-chlorophenyl) methyl carbinol, prepared from the technical product by fractional distillation under vacuum, followed by recrystallization from petroleum ether; m.p. 67–68° C. (*cf.* Grummitt, Buck and Becker, 1945).

*p,p'*DME; 1,1-bis-(*p*-chlorophenyl) ethylene, prepared by dehydration of DMC in the presence of anhydrous copper sulfate. The crude product obtained was purified by vacuum distillation, followed by recrystallization from petroleum ether and from ethanol; m.p. 84° C. (*cf.* Grummitt, Buck and Becker, 1945).

Application of benzene solutions of the chemicals was made topically by means of a micro-loop. The calibrated [1] micro-loop gave an average volume delivery of 0.00065 ml. (0.65 mm.^{3}) with less than 5 per cent error. The desired dosages were obtained by proper dilution of the solutions. The strain of flies used in this work was a multiple-resistant strain obtained from a local dairy and designated as Roberds. In a typical experiment, groups of 50 to 100 adult female flies, three to four days old, were anesthetized with carbon dioxide and the desired dosage was applied topically to the ventral thoracic region of individual flies. Following exposure, the flies were placed in holding cages provided with food and water and were kept at 26° C. and 60–70 per cent relative humidity. Mortality counts were made 24 hours after exposure.

The procedure used for studying the degradation of DDT and DMC was as follows: At a chosen interval after exposure, the flies were thoroughly rinsed in three successive 20-ml. portions of n-hexane to remove adhering particles of the chemicals. They were then ground in the presence of anhydrous sodium sulfate and extracted with carbon tetrachloride by mechanical agitation for one hour, and then filtered off. The external and internal extractions were tested for DDT and DDT-metabolites by the procedure of Schechter, Soloway, Hayes and Haller (1945). The latter procedure was also used in the determination of DMC and related compounds. The method of computation for mixtures of DDT and DDE was described in an earlier paper (Perry and Hoskins, 1951b). Since the Schechter-Haller color complexes of DDE, DMC and the ethylene derivative of

[1] Data from the Communicable Disease Center, Technical Development Branch, Public Health Service, Summary of Activities No. 25, p. 170, 1951.

DMC, 1,1-bis-(*p*-chlorophenyl) ethylene (designated as DME for convenience) have very similar absorption spectra, it is necessary to separate these compounds before quantitative determinations of each in a mixed solution can be made. To accomplish the separation of DMC and DME from DDT and DDE, use was made of a sulfuric acid-Celite column similar to that described by Davidow (1950) for the isolation of DDT from fat. A column (2.5 × 25 cm.) fitted with a glass stopcock was packed to a depth of 10 cm. The various materials dissolved in carbon

TABLE I

Separation of DDT and DDE from DMC and DME by sulfuric acid-Celite column

Number	Materials partitioned (100 microgram quantities)	Per cent recovery in eluate			
		DDT	DDE	DMC	DME
1	DDT	93.9	—	—	—
2	DDE	—	102.6	—	—
3	DMC	—	—	None	—
4	DME	—	—	—	None
5	DDT DMC	85.4	—	None	—
6	DDT DME	95.6	—	—	None
7	DDE DMC	—	96.3	None	—
8	DDT DMC DDE	96.5	96.1	None	—
9	DDT DME DDE	94.3	99.8	—	None
10	DDT DMC DME	87.1	—	None	None

tetrachloride were added in 100-microgram quantities to the columns and eluted with carbon tetrachloride until 100 ml. of eluate had been collected. It was found that DMC and DME, when chromatographed singly or in combination with DDT and DDE, were retained by the column (Table I). However, no satisfactory method was found for recovering quantitatively the DMC and DME retained by the acid-Celite mixture.

In separating the various materials from fly tissues the extracts were evaporated, redissolved in 20 ml. of carbon tetrachloride and filtered through the column.

Results

The range of mortalities of the Roberds strain resulting from topical applications of benzene solutions of *p,p'*DDT alone or in combination with *p,p'*DMC are shown in Table II. For convenience in comparing the effectiveness of the many combinations used, the data are plotted in Figure 1 as log-dosage of DDT in micrograms per fly versus mortality on the probit scale. The dosages of DMC in micrograms per fly are given on each regression line. These data permit determination of the amount of DDT required for any level of mortality with a given dosage of the synergist.

The synergistic effect resulting from separate applications of DDT and DMC was determined by pre-treating resistant flies with DMC at 6- and 24-hour in-

Table II

Twenty-four-hour mortality (per cent) of adult female house flies (Roberds strain) resulting from topical application of DDT and DMC at various ratios. Control tests showed no mortality

Micrograms fly		Per cent mortality	
DDT	DMC	Range	Average
0.32	0.65	2–14	8
	3.25	10–25	17
	6.50	20–30	25
0.65	0.06	2–20	12
	0.32	15–50	31
	0.65	40–72	53
	3.25	72–98	85
	6.50	92–98	95
1.62	0.06	10–22	17
	0.32	44–88	54
	0.65	78–98	87
	3.25	100	100
3.25	0.06	26–68	41
	0.32	68–96	86
	0.65	100	100
6.50	0.06	42–58	50
	0.32	90–96	93
	0.65	100	100
13.0	0.06	61–75	68
6.5	—	8–30	15
13.0	—	24–62	40
26.0	—	46–75	67
52.0	—	72–98	88
—	6.50	0	0
—	13.0	0	0

tervals before treatment with DDT. The reverse procedure, *i.e.*, application of DDT followed by DMC at the same intervals was also used. Simultaneous applications of DDT and synergist were used for comparison. In each case mortality counts were made 24 hours after application of the second compound. The results (Table III) indicate a marked decrease in mortality when applications of DDT and DMC were separated by 6 and 24 hours, irrespective of the order of application and as long as the dosage of DDT was kept low. With the higher dosages of DDT it is evident that application of DDT followed by DMC at the indicated intervals was more effective than the reverse procedure. This may be explained on the as-

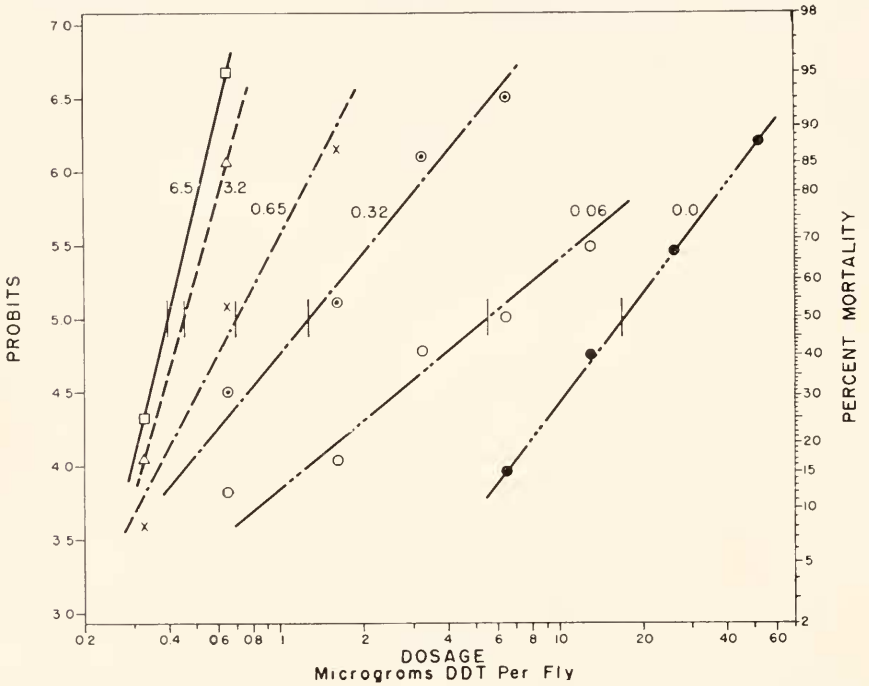


FIGURE 1. Probit-log dosage lines for adult female house flies (Roberds strain); DMC in micrograms per fly as indicated on the curves, plus various amounts of DDT applied topically to the ventral thoracic region.

sumption that sufficient unchanged DDT was still present when the DMC was applied, especially at the 6-hour interval, to produce high synergistic activity. Since in all cases simultaneous applications of DDT and DMC showed greater activity than separate applications of the chemicals, an explanation of the cause of synergistic action was sought in changes undergone by DMC after absorption.

To test this hypothesis, groups of flies were given topical applications of DMC in the usual manner. At various intervals ranging from 2 to 72 hours after exposure the flies were sacrificed and analyses of external and internal DMC were made by the procedure described earlier. Table IV shows that 24 hours after application of 13.0 micrograms DMC per fly, external rinsing removed approximately

TABLE III

Dosage-mortality data for simultaneous and separate applications of DDT and DMC at various intervals. DDT at 6.50 micrograms per fly gave an average mortality of 12 per cent. DMC had no effect at highest dosage shown

Material and dosage in micrograms/fly		Per cent mortality				
			DDT followed by DMC		DMC followed by DDT	
DDT	DMC	DDT:DMC mixture	Hours			
			6	24	6	24
0.32	3.25	26	0	0	12	2
0.65	0.65	48	0	0	6	2
0.65	3.25	82	8	0	60	4
3.25	0.32	89	80	16	24	0
6.50	0.32	96	90	58	64	10

0.4 micrograms DMC and internal extraction yielded 1.1 micrograms, giving a total of 1.5 micrograms, equivalent to 11.5 per cent recovery. Hence the undetected material, equivalent to 88.5 per cent, was either excreted or changed to some other product which did not respond to the method of analysis.

The former assumption was tested by collecting the excreta from DMC-treated flies held with food and water in covered beakers. At the end of the holding period, usually 24 hours, the flies were sacrificed and analyzed in the usual manner. The excreta from the beakers, food containers and cheesecloth covers were thoroughly washed with several portions of 2 per cent aqueous solution of sodium hydroxide followed by several rinses with n-hexane. The solvent and alkali were combined and shaken for several minutes, after which they were centrifuged and the layers separated. Analysis of the hexane portion showed the presence of only small amounts of DMC (Table V). The alkali layer was therefore acidified to pH 2–3 with 6 N sulfuric acid and extracted with ether to determine if any

TABLE IV

Amounts of external and internal DMC recovered from adult female house flies (Roberds strain) at various intervals following topical application of 13.0 micrograms DMC per fly

Interval to extraction (hours)	DMC recovered				Per cent DMC unaccounted (by difference)
	External		Internal		
	μg./fly	Per cent	μg./fly	Per cent	
2	7.65	58.8	4.50	34.6	6.6
4	5.75	44.2	6.50	50.0	5.8
8	2.05	15.7	4.46	34.3	50.0
16	0.65	5.0	1.80	13.8	81.2
24	0.55	4.2	1.48	11.4	84.4
48	0.25	2.0	0.54	4.1	93.9
72	0.17	1.3	0.50	3.8	94.9

acidic derivatives of DMC might be found. Results of four to six tests, which were averaged for each dosage (Table V), indicated the presence in the excreta of a metabolic product of DMC tentatively identified as bis-(*p*-chlorophenyl) acetic acid (DDA), on the basis of its solubility in dilute alkali. Orienting tests showed that DMC and DME are not removed by dilute alkali. None of the above products was found in extracts from untreated flies which served as controls.

From chemical considerations it appears that the conversion of DMC to DDA must proceed through the mediation of some intermediate product(s). The ease with which DMC undergoes dehydration *in vitro* suggests that the first product of degradation might be the ethylene derivative, 1,1-bis-(*p*-chlorophenyl) ethylene (DME). Tests with DME, similar to those described above for DMC, also showed the presence in the excreta of the same product, DDA (Table V). Topical applications to resistant flies of DDT-DME combinations evinced some synergistic activity, although not as marked as that with DDT-DMC. This might

TABLE V

Determination of DMC, DME and their degradation products 24 hours following topical application of DMC or DME to adult female house flies. Average of four to six tests with each dosage

Material and dosage applied in micrograms per fly	Product recovered	From tissue extracts		From excreta	
		Micrograms per fly	% of amount applied	Micrograms per fly	% of amount applied
DMC 6.5	DMC and/or DME	0.69	10.7	0.32	4.8
	DDA	0.62	9.5	4.20	64.6
DMC 13.0	DMC and/or DME	1.62	12.5	0.66	5.0
	DDA	0.84	6.4	6.80	52.3
DME 13.0	DME	1.30	10.0	0.18	1.4
	DDA	0.70	5.4	6.50	50.0

also serve as an indication of the intermediate role played by DME in the metabolism of DMC. In addition to DME, it is conceivable that one or possibly more intermediate(s) are formed in the conversion of DMC to DDA.

The degradation of DDT to the relatively nontoxic derivative DDE has been shown by Sternburg, Kearns and Bruce (1950). Perry and Hoskins (1950, 1951b) and Fullmer and Hoskins (1951) further demonstrated the inhibiting action of piperonyl cyclonene on the degradation of DDT. Winteringham, Loveday and Harrison (1951) obtained the same results with the bromine analog of DDT. If ability of resistant flies to survive is, in part, a function of detoxification of DDT, then inhibition of this process should show a quantitative relationship to mortality.

To obtain quantitative data on this subject, a series of tests was designed to determine: (1) the amounts of DMC in combination with a fixed dosage of DDT required to produce varying levels of mortality; (2) the effect of these amounts of DMC upon the degradation of DDT; and (3) the correlation, if any, between inhibition of detoxification and mortality. The results of three tests with groups of

100 flies per test shown in Table VI make such a correlation possible. Per cent inhibition was calculated as follows:

$$\frac{\text{Amount of DDE recovered from application of DDT and DMC}}{\text{Amount of DDE recovered from application of DDT alone}} \times 100 = X$$

$100 - X =$ per cent inhibition.

It may be noted that as the dosage of DMC was increased there was a corresponding increase in per cent inhibition of DDE formation and, consequently, an increase in mortality. It is also evident that complete inhibition was not essential for 100 per cent mortality of resistant flies.

TABLE VI

The effect of DMC on inhibition of conversion of DDT to DDE and on mortality of adult female resistant house flies. Extractions made 24 hours after application. DMC separated chromatographically

Applied micrograms/fly		Recovered*				Per cent inhibition	Per cent mortality
		DDT		DDE**			
DDT	DMC	μg./fly	% of DDT applied	μg./fly	% of DDT applied		
0.65	0.0	0.0	0.0	0.51	78.4	—	0.0
0.65	0.06	0.06	9.4	0.41	63.0	19.6	2.0
0.65	0.13	0.11	16.9	0.38	58.4	25.5	14.0
0.65	0.32	0.18	27.7	0.32	49.2	37.2	25.0
0.65	0.65	0.29	44.6	0.26	40.0	49.0	50.0
0.65	1.30	0.35	53.8	0.18	27.7	64.7	72.0
0.65	3.25	0.45	69.2	0.12	18.4	76.5	92.0
0.65	6.50	0.50	76.9	0.08	12.3	84.3	100

* External rinses showed no DDT or DMC present.
** DDE figures are expressed as molecular equivalents of DDT.

DISCUSSION

Synergism has been defined in many different ways; however, most definitions agree on one common point, *i.e.*, that the physiological effect of the substances in a mixture is significantly greater when used together than the summation of their individual effects. Bliss (1939) suggests that when the constituents of a mixture act independently, its toxicity does not depend upon the relative proportions of the components but only upon their inherent potencies. Synergistic action, in contrast, involves the ratio of one component to the other.

A conspicuous feature of Figure 1 is that as increasing amounts of DMC are added to DDT the probit-log dosage lines move toward smaller dosages of DDT and, in addition, become successively steeper. This process continues until a point is reached beyond which further addition of the synergist, within limits, causes either no increase or a slight decrease in activity. As seen from Figure 2, the addition of a small amount of DMC caused a significant drop in the amount of DDT required for a given mortality, whereas with large dosages of the adjuvant the effect was much less pronounced, as indicated by the hyperbolic nature of the curves. This reduction in DDT was roughly proportional to the mol fraction of DMC over

a limited range of dosages, until the DMC was present in approximately equimolecular proportions with DDT. Figure 2 indicates that but little could be gained by further addition of DMC beyond this point; hence, by visual inspection, it may be reasonable to suppose that the 1:1 ratio is the optimum ratio of DDT:DMC.

It may also be noted (Fig. 1) from the data for the LD_{50} and the LD_{90} with DDT alone, and those for DDT plus the maximum amount of DMC, that the resistance of the Roberds strain may, under experimental laboratory conditions, be reduced to the extent of about 98 per cent (from 17.0 gamma to 0.40 gamma DDT per fly for the LD_{50}, and from 56.0 gamma to 0.58 gamma per fly for the LD_{90}). It should be borne in mind that these values are calculated in terms of amounts of

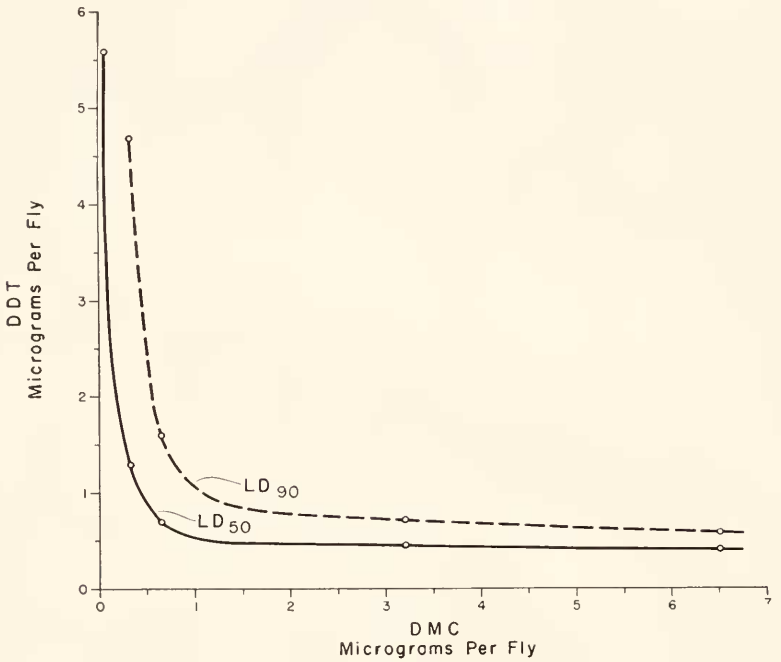


FIGURE 2. Curve relating dosage of DDT with dosage of DMC for 50 per cent and 90 per cent mortality of adult female house flies (Roberds strain).

DDT applied. Since the rate of absorption of DDT by house flies does not increase proportionally to increasing dosages by topical application (Fig. 3), the percentage reduction of resistance, if calculated on the basis of DDT absorbed, would be somewhat lower than 98 per cent. In no case, however, was it possible to reduce the resistance of the Roberds strain completely to the level of the susceptible strain (LD_{50} approximately 0.30 microgram per fly).

There is reason to believe that synergistic compounds do not all act in the same manner, nor do they affect the same physiological system *in vivo*. It has been shown by various workers (Lindquist, Madden and Wilson, 1947; Wilson, 1949; Chamberlain, 1950; Perry and Hoskins, 1951a; and others) that pre-treating house flies with synergists followed by application of pyrethrins or DDT at different

intervals did not materially affect the resultant mortalities as compared with simultaneous application of both compounds. In the present case, however, a marked reduction in activity resulted when applications of DMC and DDT were separated (Table III). This may indicate a different mechanism of synergistic action from those mentioned above.

The data of Tables IV and V show that DMC is rapidly metabolized by living flies. Thus, in 24 hours, from 50 to 70 per cent of the amount of DMC applied is excreted as DDA, and from 10 to 15 per cent is retained in the body either un-

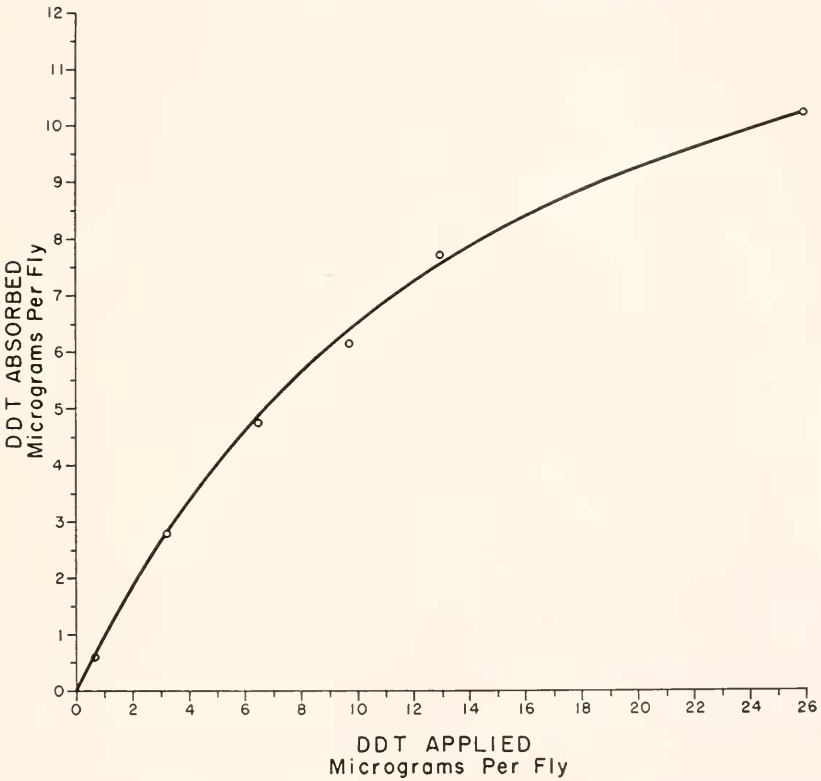


FIGURE 3. Curve relating amount of DDT absorbed in 24 hours with amount of DDT applied to adult female house flies (Roberds strain).

changed or in the form of DME. When sub-lethal amounts of DDT-DMC combinations are applied, both DDE and DDA are formed in substantial quantities. Lethal dosages of DDT-DMC, on the other hand, yield large amounts of DDT, small amounts of DDE and only traces of DDA. As a typical example, application of 0.3 gamma DDT and 0.3 gamma DMC per fly yielded in 24 hours 0.07 gamma DDT, 0.16 gamma DDE and 0.18 gamma DDA per fly, and the resulting mortality was 0–10 per cent. On the other hand, application of 0.6 gamma DDT plus 2.4 gamma DMC per fly yielded 0.36 gamma DDT, 0.13 gamma DDE and only traces of DDA, while the resulting mortality was 70–90 per cent.

Since no satisfactory analytical methods for distinguishing between DMC and

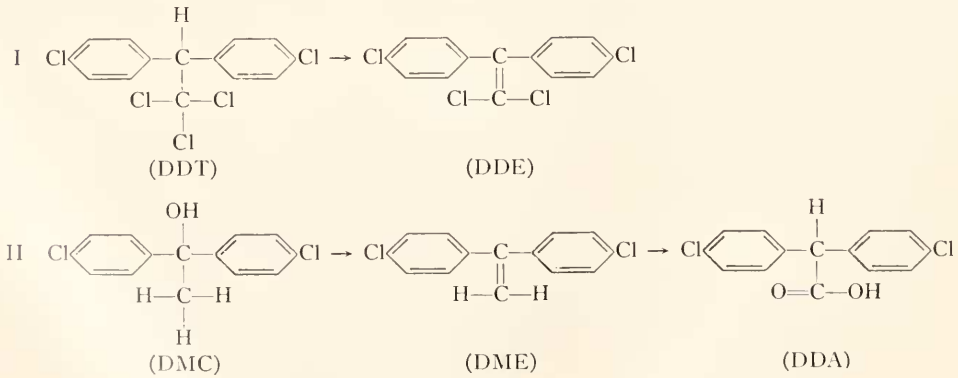
DME are available, the role of DME in intermediary metabolism cannot be ascertained. However, on the basis of the following considerations, namely, (a) the relative ease of dehydration of DMC *in vitro*, (b) the conversion of DME to DDA *in vivo*, and (c) the fact that DME exhibits some synergistic activity with DDT, it is postulated that DME might be an intermediate product of DMC metabolism.

In attempting to elucidate the mechanism of synergistic action of DMC it is assumed that dehydrohalogenation of DDT, dehydration of DMC, as well as formation of DDA are enzymatic processes. As shown in Table VI a correlation exists between the extent of synergistic activity of DMC, as measured in terms of mortality, and the degree of inhibition of DDE formation.

Inhibitors of enzymatic reactions are usually of two types, competitive and non-competitive. In non-competitive inhibition, inactivation of the enzyme is independent of substrate concentration and depends only on concentration of inhibitor. In competitive inhibition, the inhibitor competes with the substrate for specific groups of the enzyme so that the apparent decrease in enzyme activity depends on the relative concentrations of both substrate and inhibitor (Lardy, 1949). In the present case it is apparent that inhibition is not of a non-competitive type, for the degree of activity is not independent of substrate (DDT) concentration (Fig. 1). Thus, for any constant dosage of inhibitor (DMC) the activity increased with increasing amounts of added substrate. Likewise, increased activity was manifested when increasing amounts of inhibitor were added to a constant substrate dosage.

The great structural similarity between DMC and DDT and the knowledge that DMC is metabolized by living flies, plus the fact that greatest synergistic effect is manifested when DMC and DDT are applied simultaneously, suggest that inhibition is of a competitive type. This concept of "competition by displacement" is unlike the type of competitive inhibition by structurally related analogs of essential metabolites in which the added substrate may counteract the action of the inhibitor. In the present case the so-called metabolite or substrate is a poison which resistant flies are able to detoxify in varying quantities. Clearly, the addition of increasing amounts of this substrate cannot nullify the action of the inhibitor, but on the contrary, can only contribute to the injury already done.

The postulated reactions in this competitive system may be summarized as follows:



The quantitative relationship between inhibition of DDE formation and mortality of resistant house flies (Table VI) indicates that detoxification of DDT is a major factor in survival. If this were not the case, it would be difficult to explain why resistant flies should succumb to small quantities of DDT-DMC mixtures when they are able to tolerate large dosages of DDT.

DMC is non-toxic to house flies at fairly high dosages, and no observable symptoms are noted from application of DMC alone. Unless DMC causes the derangement of an unknown physiological process in the fly which might be associated with resistance, the above data point toward detoxification of DDT as a major factor in survival, and to inhibition of this process by DMC as a factor in mortality.

The nature and properties of the DDT-detoxifying mechanism have not yet been ascertained. However, the work of Sacktor (1951a, 1951b) on cytochrome oxidase in resistant and susceptible house flies suggests that this enzyme system might be associated with DDT-resistance. The relationship, if any, between cytochrome oxidase, DDT-detoxification and the inhibiting action of synergists has not been studied.

It is believed that the data presented in this report lend support to the hypothesis manifesting a specific competitive type of inhibition of DDT-detoxification as the mode of action of DMC as a DDT synergist.

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Summary

1. DMC has no insecticidal properties but markedly enhances the effectiveness of DDT against resistant house flies. The addition of a small amount of DMC causes a significant drop in the amount of DDT required for a given mortality.

2. Data interpolated from probit-log dosage lines for DDT alone and DDT plus DMC show that, under experimental laboratory conditions, the resistance of the Roberds strain can be markedly reduced.

3. Greatest synergistic effect is manifested when DMC and DDT are applied together, for separate application of the chemicals at 6-hour and 24-hour intervals shows a marked reduction in mortality.

4. DMC is rapidly metabolized by living flies and is excreted principally as a product tentatively identified as bis-(*p*-chlorophenyl) acetic acid (DDA). The compound 1,1-bis-(*p*-chlorophenyl) ethylene is suggested as being an intermediate product in DMC metabolism.

5. A correlation is shown between the extent of synergistic action and the degree of inhibition of DDT-detoxification in resistant flies.

6. Inhibition appears to be of a competitive type, the synergist competing with the insecticide for the mechanism of detoxification.

LITERATURE CITED

Bliss, C. I., 1939. The toxicity of poisons applied jointly. *Ann. Appl. Biol.*, **26**: 585–615.

Chamberlain, R. W., 1950. An investigation on the action of piperonyl butoxide with pyrethrum. *Amer. J. Hygiene*, **52**: 153–183.

David, W. A. L., and P. Bracey, 1944. Activation of pyrethrins in fly sprays. *Nature,* **153**: 594–595.

Davidow, B., 1950. Isolation of DDT from fat. *J. Official Agr. Chemists,* **33**: 130.

Dove, W. E., 1947. Piperonyl butoxide, a new and safe insecticide for the household and field. *Amer. J. Trop. Med.,* **27**: 339–345.

Fullmer, O. H., and W. M. Hoskins, 1951. Effects of DDT upon the respiration of susceptible and resistant house flies. *J. Econ. Ent.,* **44**: 858–870.

Grummitt, O., A. C. Buck and E. I. Becker, 1945. 1,1-Di-(*p*-chlorophenyl)-ethane. *J. Amer. Chem. Soc.,* **67**: 2265.

Lardy, H. A., 1949. Respiratory enzymes. Burgess Publishing Co., Minneapolis, Minn.

Lindquist, A. W., A. H. Madden and C. H. Wilson, 1947. Pretreating house flies with synergists before applying pyrethrum sprays. *J. Econ. Ent.,* **40**: 426–427.

Marcii, R. B., R. L. Metcalf and L. L. Lewallen, 1952. Studies on synergists for DDT against resistant house flies. *J. Econ. Ent.,* **45**: 851–860.

Munro, J. W., 1942. The entomology of commerce; insect pests and their control. *Analyst,* **67**: 155.

Page, A. B., and R. E. Blackith, 1949. Bioassay systems for pyrethrins, II. Mode of action of pyrethrum synergists. *Ann. Appl. Biol.,* **36**: 244–249.

Parkin, E. A., and A. A. Green, 1944. Activation of pyrethrins by sesame oil. *Nature,* **154**: 16–17.

Perry, A. S., and W. M. Hoskins, 1950. The detoxification of DDT by resistant house flies and inhibition of this process by piperonyl cyclonene. *Science,* **111**: 600–601.

Perry, A. S., and W. M. Hoskins, 1951a. Synergistic action with DDT toward resistant house flies. *J. Econ. Ent.,* **44**: 839–850.

Perry, A. S., and W. M. Hoskins, 1951b. Detoxification of DDT as a factor in the resistance of house flies. *J. Econ. Ent.,* **44**: 850–857.

Sacktor, B., 1951a. A comparison of the cytochrome oxidase activity of two strains of house flies. *J. Econ. Ent.,* **43**: 832–838.

Sacktor, B., 1951b. Some aspects of respiratory metabolism during metamorphosis of normal and DDT-resistant house flies, *Musca domestica* L. *Biol. Bull.,* **100**: 229–243.

Schechter, M. S., S. B. Soloway, R. A. Hayes and H. L. Haller, 1945. Colorimetric determination of DDT. Color test for isolated compounds. *J. Ind. Eng. Chem., Anal. Ed.,* **17**: 704–709.

Sternburg, J., C. W. Kearns and W. N. Bruce, 1950. Absorption and metabolism of DDT by resistant and susceptible house flies. *J. Econ. Ent.,* **43**: 214–219.

Sumerford, W. T., Mary B. Goette, K. D. Quarterman and Sheely L. Schenck, 1951. The potentiation of DDT against resistant house flies by several structurally related compounds. *Science,* **114**: 6–7.

Sumerford, W. T., R. W. Fay, Mary B. Goette and A. Marian Allred, 1951. Promising DDT-synergist combinations for the control of resistant flies. *J. Nat. Malaria Soc.,* **10**: 345–349.

Wilson, C. S., 1949. Piperonyl butoxide, piperonyl cyclonene, and pyrethrum applied to selected parts of individual flies. *J. Econ. Ent.,* **42**: 423–428.

Winteringham, F. P. W., Patricia M. Loveday and A. Harrison, 1951. Resistance of house flies to DDT. *Nature,* **167**: 106.